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A Methodology for Assessing the Impact of Mutagens on Aquatic Ecosystems

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Introduction

Assessments of impacts of hazardous agents (i.e., chemical and physical mutagens) on human health have focused on defining the effects of chronic exposure on individuals, with cancer being the main effect of concern. In contrast, impacts on ecosystems have traditionally been gauged by the assessment of near-term organism mortality, which is clearly not a useful endpoint for assessing the long-term effects of chronic exposures. Impacts on individual organisms that affect the long-term survival of populations are much more important but are also more difficult to define. Therefore, methods that provide accurate measures of sub-lethal effects that are linked to population survival are required so that accurate assessments of environmental damage can be made and remediation efforts, if required, can be initiated.

Radioactive substances have entered aquatic environments as a result of research and production activities, intentional disposal, and accidental discharges. At several DOE sites, surface waters and sediments are contaminated with radioactive and mutagenic materials. At the Oak Ridge National Laboratory (ORNL), for example, ^{60}Co , ^{137}Cs , and ^{90}Sr have been detected at elevated levels in bluegill fish collected from watersheds on the site (Kornegay *et al.* 1991). At the Savannah River Site (SRS), fish sampled off site in the Savannah River contain measurable concentrations of alpha activity and ^{137}Cs (Cummins *et al.* 1991). The accident at the Chernobyl power station in the former Soviet Union (FSU) has resulted in the contamination of biota present in the Kiev Reservoir. In addition, ocean disposal of

radioactive materials by the FSU in the Arctic has led to recent concerns about impacts on aquatic biota.

The impacts of low-level releases of radioactive materials into aquatic ecosystems are largely unknown because methods are not available that can be used to link environmental levels of radiation with impacts on populations of aquatic organisms. This gap in knowledge has occurred because most previous studies of biological effects relied on analytical and toxicological methods that were not sensitive enough to detect the sublethal effects caused by the relatively low levels of radiation that are present in the environment. Furthermore, information available on the effects of radiation on responses of aquatic organisms is primarily on determinations of mortality rates and histopathology. The extensive data on the effects of acute radiation on mortality rates in aquatic animals appear to indicate that radio sensitivity increases with biological complexity, i.e., that the higher the phylogenetic position, the lower the LD₅₀ (Woodhead 1984; Blaylock and Trabalka 1978; Templeton 1976). However, more recent data on effects of acute radiation at the cellular level indicate that this conclusion may not be valid. For example, genetic damage (i.e., chromosomal aberrations and sister chromatid exchanges) caused by acute radiation in the marine polychaete *Neanthes arenaceodentata* occurred at doses that did not differ greatly from doses inducing such responses in some mammals (Anderson and Harrison, 1986, 1990; Harrison *et al.*, 1987; UNSCEAR 1986).

Technical Approach

To address the effects of a direct-acting mutagen (i.e., radiation) on aquatic organisms, we have used a methodology that applies sensitive state-of-the-art techniques for assessing damage to genetic material. Our long-term objectives are to develop and apply techniques that can be used to determine the effects of radiation on organisms obtained from contaminated environments so that impacts on the reproductive success of impacted populations can be assessed. To meet this goal, we have coupled dosimetry measurements with determinations of sensitive biological effects (i.e., damage to genetic material).

Methods that are specific for determining radiation-induced genetic damage in the amphipod, *Ampelisca abdita*, and in the blue mussel, *Mytilus edulis* were developed. During the course of this work, however, the U.S.

EPA expressed an interest in using marine bivalves as sentinels for detecting the extent of radioactive contamination in the Arctic seas. The focus of this work, therefore, was subsequently directed toward defining the suitability of using *M. edulis* as such a sentinel species.

For the assessment of genetic damage, adult organisms received a range of doses of radiation. Cesium-137, a gamma emitter, was used as a point source to provide acute irradiations of whole animals to determine the dose-response relationship for DNA single-strand breaks. A sealed ^{137}Cs source (4,200 Ci; J.L. Shepherd and Associates) was used to irradiate the test organisms. Mussels received acute irradiations in 400-mL glass beakers that contained three mussels and 300 mL of 0.22-mm-filtered water. The dose rate employed was approximately 4.5 Gy min^{-1} . Organisms were exposed to a series of radiation doses from 0.05 to 1.0 Gy. Gill tissues (0.10 to 0.30 g) were subsequently removed from control and radiation-exposed groups and were.

Following acute exposures, genetic damage was assessed through the use of a DNA single-strand break assay (Martinelli 1991; Martinelli *et al.* 1989, 1994; Nacci and Jackim 1989; Shugart 1988a,b; Kohn *et al.* 1976). This assay takes advantage of the fact that DNA strand separation takes place at single-strand breaks that occur as a result of damage due to chemical and physical agents. This damage was quantified by staining the DNA molecule with a fluorescent dye (Hoechst 33258) and monitoring the complex in a DNA fluorimeter at 450 nm (Downs and Wilfinger, 1983). Damage to DNA can be quantified because normal, double-stranded DNA (dsDNA) has approximately twice the fluorescence of damaged, single-stranded DNA (ssDNA).

Thermoluminescent dosimeters (TLDs) were used to establish the doses received by the test organisms in the laboratory. A TLD package that consisted of three TLDs that were separated by plastic and were wrapped in one layer of aluminum foil were placed in the shells from which the soft body tissues had been removed. In this way, the attenuation of the dose by the glass beaker, water, and bivalve shells was determined. Control groups of mussels were handled in an identical fashion with the exception of irradiation.

Following irradiation, the exposed and control organisms were dissected immediately, and the integrity of the DNA was determined. The amounts of ds- and ss-DNA were then determined in the DNA sample that was isolated as described above. Triplicate analyses were conducted for each organism. The extent of DNA strand breakage was subsequently determined by calculating an F value (i.e., [ouDNA - ssDNA]/[dsDNA - ssDNA]) for each exposure and control group. Differences in control and exposure groups were determined statistically using one-factor ANOVA, the F-test, and the student's t-test.

A major thrust of this study was to increase the sensitivity of the DNA single-strand break assay so that DNA damage at radiation doses ≤ 0.10 Gy can be detected. A high-performance liquid chromatography (HPLC) system coupled with fluorescence detection capabilities was assembled and used to resolve and quantify single- and double-stranded DNA. The application of this approach was intended to increase the sensitivity of this assay by isolating the fluorescence associated with the single-stranded DNA fragments, which in the conventional method, are masked by the fluorescence of the double-stranded DNA. The HPLC consisted of an Altex Model 334 system (Beckman Instruments, Inc., San Ramon, CA) that was equipped with a fluorescence detector and a fifty- μ L injection loop. Separation of DNA fragments from dsDNA was accomplished on a Bio-Gel HPHT column (Bio-Rad Laboratories, Richmond, CA). Analyte elution was accomplished with a phosphate buffer mobile phase, which was run at flow rate of 1.0 mL/min and at a gradient of increasing strength (0.10 to 0.30 M). Detection of analytes was accomplished via excitation at 220 nm and detection of fluorescence at 450 nm.

Results and Discussion

Attenuation of radiation delivered to the bivalves from the ^{137}Cs source was low. The relationship between nominal and delivered gamma radiation doses as determined by TLD measurements is presented in Figure 1. There was not a significant difference between radiation attenuation by the dosimeter package alone and attenuation by the bivalve shell. These results indicate that TLDs can be used to provide accurate dosimetry information for assessing exposure to gamma radiation.

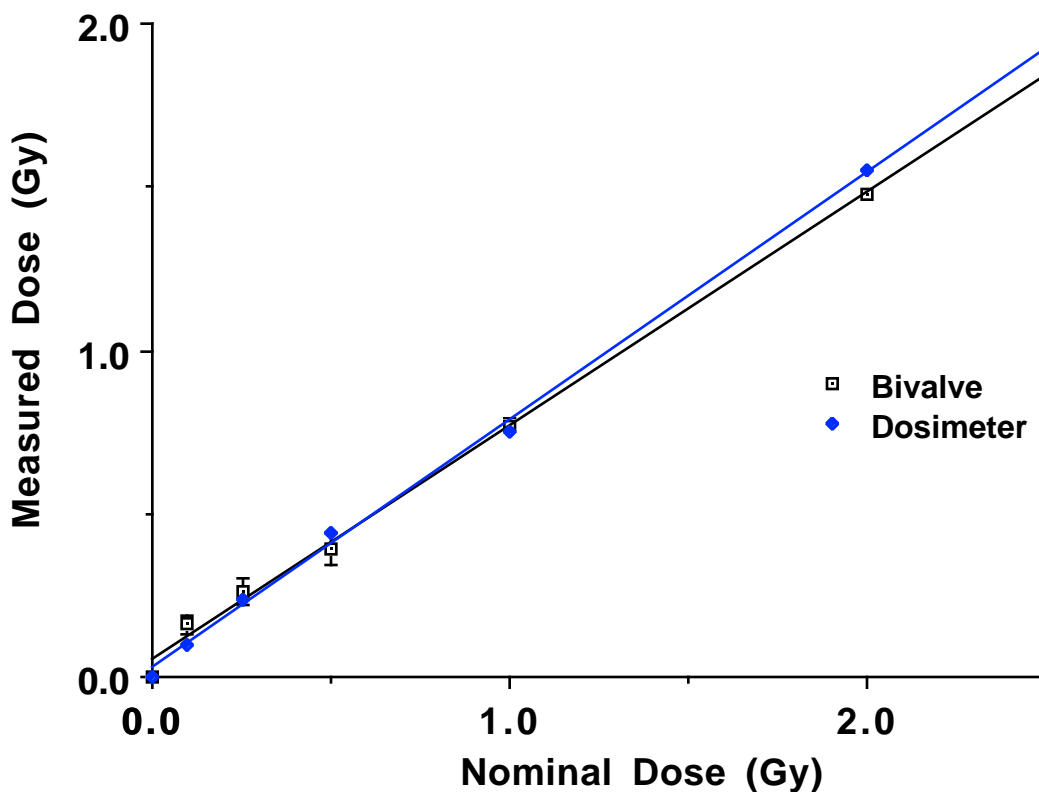


Figure 1. The relationship between nominal and delivered doses for thermoluminescent dosimeter packages exposed to gamma radiation directly or inside bivalve shells.

The use of the HPLC-fluorescence system for the detection of DNA single-strand breaks was found to be less sensitive than the standard method. The quantification of non double-stranded DNA was limited by the variability in fluorescence that occurs due to the presence of non-uniform DNA-Hoechst complexes. Similar results have been reported recently by Theodorakis *et al.* (1994) who used gel electrophoretic techniques to assess DNA strand breaks in fish that resulted from exposure to chemical toxicants. Their results reveal similar difficulty with DNA-strand resolution. Accordingly, the standard fluorimetric technique, which provides reproducible results with a loss in sensitivity, was used to assess DNA damage in subsequent experiments.

Bivalves that were exposed to ^{137}Cs exhibited increased percentages of DNA strand breaks. A direct relationship between the percent of single-stranded DNA and radiation dose was observed (Fig. 2). Significant effects ($p < 0.001$) were detected at the lowest delivered dose (0.16 ± 0.03 Gy).

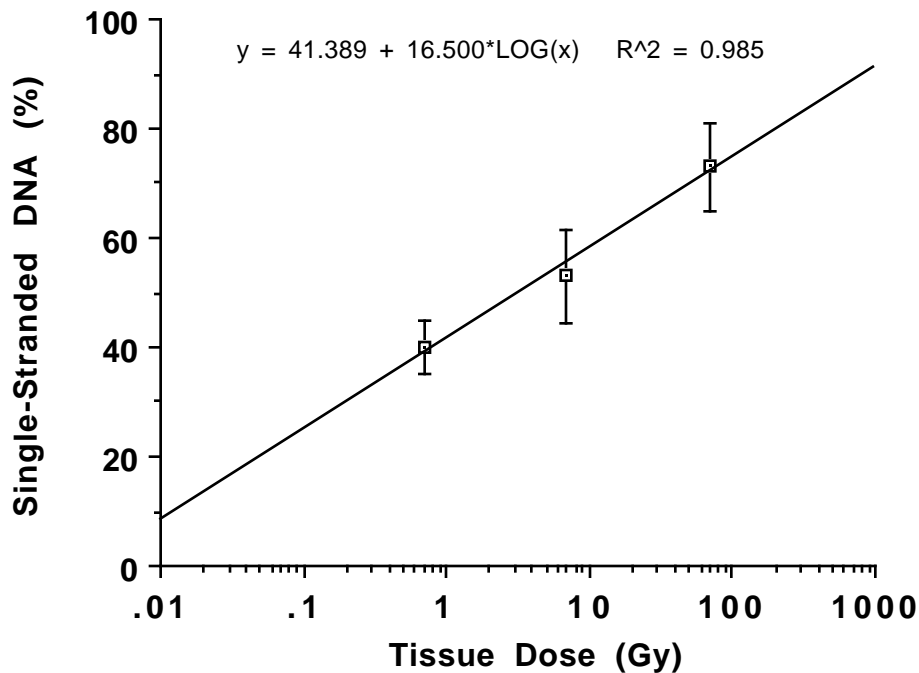


Figure 2. The relationship between single-stranded DNA and radiation dose in *Mytilus edulis*. Tissue doses were determined from thermoluminescent dosimeters.

The results from this study compare well with other studies (Table 1) and indicate a significant improvement in sensitivity for the detection of genetic damage. Previous research on radiation effects on genetic material in aquatic organisms, however, has focused on techniques that require the visualization of individual chromosomes. Such methods are relatively time consuming and costly and can only be performed on organisms with appropriate karyotypes (i.e., those with small numbers of large chromosomes).

Impacts on the genetic material in aquatic organisms may result in reduced fertility if the damage to DNA induces cell death in dividing gametes. In addition, reproductive success may be impaired if in the chromosomes

dominant- and recessive-lethal mutations are induced, causing embryo mortality or abnormality. Recent work has shown that alterations of genetic material and decreases in reproductive capacity are not only sensitive indicators of radiation effects but, in some aquatic organisms, are induced in the same dose range (see Table 1). The results of these studies indicate that assessments of damage to genetic material may provide an indication of the potential for impacts on reproductive in exposed populations. Ultimately, the linkage of these genotoxic effects to population survival are required so that accurate assessments of ecosystem damage can be made.

Table 1. The relationship between radiation dose and biological effects in aquatic organisms. Unless otherwise noted, data are for the marine polychaete, *Neanthes arenaceodentata*.^a

Dose (Gy)	Effect (life stage)
500	Mortality (adults)
100	Reduction in life span (adults)
10	Reduction in brood size (adults)
2.0	Increased chromosomal aberrations (larvae)
0.60	Increased SCE ^b frequency (larvae)
0.50	Decreased % live embryos in brood (adults)
0.16	Increased % single-stranded DNA (adults) ^c

^a Except as noted, data are from Anderson *et al.* (1990) and Harrison and Anderson (1994).

^b Sister chromatid exchange.

^c For *M. edulis* (this study).

Future Work

The results of this study have laid a foundation for obtaining continued funding from outside sources. Future work will be directed toward the development of a tiered system to assess the impacts of radiation on genetic material and reproductive success for critical indicator species. By determining in the same population of organisms sublethal biological effects (genetic damage and reproductive effects) and radiation dosimetry, the information obtained can be used to set realistic radiation-exposure limits to

protect ecosystems from planned or accidental releases of radioactive materials into aquatic environments.

The EPA's Office of Radiation Programs has an expressed interest in using this approach to assess ecological impacts of radiation, especially in the Arctic seas. In collaboration with the EPA, a proposal has been submitted to the Office of Naval Research entitled "Sentinel Systems for the Assessment of Radiation-Induced Ecological Impacts in the North and Pacific Arctic Oceans," which builds on the results of this study. In addition, the EPA Office of International Affairs is in the process of brokering a funded collaborative effort with Polish scientists who are using electron paramagnetic resonance spectroscopy (EPR) to measure radiation doses in bivalve shells. Our work on biological effects will be used to correlate these dosimetric measurements with biological effects.

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